GAGE (S. H.)

permanent microscopic preparation of Amphibian blood –

(*From the American Naturalist, October*, 1880.)

PERMANENT MICROSCOPIC PREPARATIONS OF AMPHIBIAN BLOOD. —The very excellent method of drying the corpuscles of mammalian blood on the microscopic slide, is not applicable to the much more bulky corpuscles of Amphibia. The corpuscles of the latter are sure to be distorted and seamed in drying; hence various methods of preserving the corpuscles moist have been tried with varying success.

The following very great modification of the method proposed by Ranvier in his treatise on histology,[1] has been in use for some time in the Anatomical Laboratory of Cornell University, and has given uniformly excellent results. Preparations made three years ago are quite as good as at first.

Three or four drops of fresh blood are allowed to fall into 10 cc. of normal salt solution (common salt 750 milligrams, water 100 cc.) preferably contained in a high narrow vessel like a graduate glass or beaker. The mixture of blood and salt solution should be well agitated and then 100 cc. of a saturated aqueous solution of picric acid added with constant stirring. After the corpuscles have settled, as much of the supernatant liquid as possible is poured off, and in its place is put about an equal volume of normal salt solution. The corpuscles are allowed to settle, the liquid poured off and another volume of salt solution added. This is continued until the salt solution acquires only a faint yellow tinge.

The use of the salt solution is, first, to dilute the blood in order to avoid distortion of the corpuscles, and second, to wash away the picric acid so that the subsequent staining will be more satisfactory.

After pouring off the last salt solution, there is put in its place 10 cc. of a mixture of five parts of Frey's carmine and ninety-five parts of picrocarmine. The corpuscles will stain in from one to fifteen hours. A drop of the agitated mixture should be examined occasionally to ascertain when the staining is sufficient. The nucleus should be deep red, and the body of the corpuscle yellow or pinkish.

When the staining is completed, as much stainer as possible should be poured off, and in its place 10 or 15 cc. of acid glycerine (glycerine 100 cc., acetic or formic acid 1 cc.). This mixture of corpuscles and glycerine may be placed in a bottle and used at any time, it being simply necessary to agitate the mixture slightly or to take up some of the sediment with a pipette and mount it precisely as any other glycerine preparation.

Summary.—1. The fresh blood is first diluted with about fifty times its volume of normal salt solution.

2. To this diluted blood is added ten times as great a volume of a saturated aqueous solution of picric acid.

3. The picric acid is washed away with normal salt solution.

[1] Traité technique de Histologie, p. 195.

4. The corpuscles are stained with picrocarmine, or a mixture of this and Frey's carmine.

5. They are preserved in acid glycerine, and may be mounted for the microscope at any time.—*Read at the sub-section of Microscopy of the A. A. A. S., by Simon H. Gage, Ithaca, N. Y.*